Office Action Summary	Application No.	Applicant(s)	
	10/798,790	TOTEY ET AL.	
	Examiner	Art Unit	
	DANIEL C. GAMETT	1647	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *21 February 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *5-9,12-33 and 38* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *5-9,12-33 and 38* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☒ Interview Summary (PTO-413) Paper No(s)/Mail Date. *6/21/08* .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. The amendments of 02/21/2008 have been entered in full. Claims 1-4, 10, 11, 34-37, and 39-44 are cancelled. Claims 5-9, 12-33, and 38 are under examination.
2. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claim Rejections - 35 USC § 103

3. Claims 5-9, 12-33, and 38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rolletschek *et al.*, Mechanisms of Development (2001) 105:93-104, as applied to claims 33 and 34 above, and further in view of WO 200188104, published November 22, 2001 (Carpenter 2001) and US 20020068045, (Reubinoff) published June 6, 2002 (all references of record).
4. In the office action mailed 09/21/2007, these same references were cited in a rejection of claims 5-8, 12-32, 35, 36, and 38. The rejection of record established that Rolletschek *et al.* derived nestin+ neuroprogenitor cells from mouse embryonic stem cells and teach that TGF-β3 and interleukin-1β promote differentiation of the cells to yield a population with >40% dopaminergic neurons. Furthermore, culturing pluripotent embryonic stem cells to form embryoid bodies followed by culture in serum-free ITSFn medium to select nestin+ neural stem cells (instant claims 12-22), as well as expansion and differentiation of the selected neural progenitor cells in the presence of insulin, selenite, transferrin, putrescine, GDNF, dbcAMP (instant claims 26-28 and 31) are well known in the art, as evidenced by

Rolletschek *et al.* in section 4.1, page 101. Rolletschek *et al.* passaged and cultivated selected cells for greater than 30 days (as in instant claims 27, 28, and 32; see Rolletschek *et al.* Figs. 2-6). Carpenter and Reubinoff teach the derivation of neural stem cells from human embryonic stem cells, and teach that such cells are positive for both nestin and NCAM. Carpenter teaches selection of neural stem cells from human embryonic stem cells via embryoid body (EB) formation followed by cultivation and differentiation in a serum-free medium supplemented with fibronectin, bFGF and neurotrophic factors (as in instant claims 12, 13, 18, 26, 31) and magnetic bead sorting of NCAM+ cells (see Example 1, Table 3; Table 8; Figs. 1 and 3); the selected cells expressed nestin (Table 8, p. 26) and could differentiate into dopaminergic neurons (Fig. 4). Likewise, Reubinoff teaches that human embryonic stem cells cultured in serum-free medium (which permits embryoid bodies to form) differentiate into neural cells that are positive for both nestin and NCAM (see Fig. 5 and [0067]); the cells further differentiate into neurons (including TH+ neurons) and glia (Figs. 28 and 29). Reubinoff further teaches the common practices of cryopreserving cells (as in instant claim 29) and [0290] and laser dissection of blastocysts at (as in instant claims 6 and 8) at [0136]. As either nestin or NCAM can be used to identify neural stem cells, it is obvious to use them both to provide further enrichment from a mixed population. While mouse and human pluripotent embryonic stem cells are notoriously different in their growth and differentiation requirements, the target for TGF-β3 and interleukin-1β taught in Rolletschek is not a pluripotent embryonic stem cell, but a multipotent, nestin positive neural stem cell. Therefore, one of skill in the art would have a reasonable expectation of success in achieving differentiation of dopaminergic and serotonergic neurons by selecting nestin and

NCAM positive cells from a population of human pluripotent cells, as taught by Carpenter and Reubinoff, and cultivating the selected cells in the presence of TGF-β3 and interleukin-1β, as taught in Rolletschek, to arrive at the generic methods of the instant claims.

5. The rejection of record also stated that the specific high yields recited (at that time) in instant claims 9, 10, 37, and 39 appear to be unprecedented and unexpected, therefore these claims were not rejected. Upon further consideration, however, the "wherein" expressions which now appear in claims 5, 9, 33, and 38, do not distinguish over the art because they simply expresses the intended result of a process step positively recited (see MPEP 2111.04). That is, the claims as amended recite steps that are well known in the art to be useful for deriving differentiated neurons from human embryonic stem cells, but then attempt to distinguish over the art by simply asserting an unprecedented outcome. The expression of an intended outcome does not alter the way in which the method is performed. The claims do not recite the disclosed method that produced the unexpected result.

Claim Rejections - 35 USC § 112

6. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

7. Claims 5-9, 12-33, and 38 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of generating a differentiated neural cell population from primate pluripotent stem cells wherein the differentiated neural cell

population comprises at least about 60% dopaminergic neurons or at least about 30% serotonergic neurons, comprising the steps (a) expanding a culture of primate pluripotent stem cells; (b) forming embryoid bodies and selecting for neuroprogenitor cells that are positive for nestin by culturing the pluripotent stem cells in serum-free medium comprising insulin, sodium selenite, transferrin, and fibronectin (c) sorting the nestin-positive neuroprogenitor cells for enrichment of NCAM-positive cells; (d) differentiating the nestin-positive, NCAM-positive cells by culturing the cells in a differentiation media which comprises TGF-β3 or interleukin-1β, or both, does not reasonably provide enablement for any method that omits the steps of embryoid body formation or culturing the pluripotent stem cells in serum-free medium comprising insulin, sodium selenite, transferrin, and fibronectin. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention commensurate in scope with these claims.

8. The courts have interpreted the first paragraph of 35 U.S.C. 112 to mean that the specification must enable one skilled in the art to make and use the invention without undue experimentation. The courts have further interpreted undue experimentation as requiring "ingenuity beyond that to be expected of one of ordinary skill in the art" (Fields v. Conover, 170 USPQ 276 (CCPA 1971)) or requiring an extended period of experimentation in the absence of sufficient direction or guidance (In re Colianni, 195 USPQ 150 (CCPA 1977)). Additionally, the courts have determined that "... where a statement is, on its face, contrary to generally accepted scientific principles", a rejection for failure to teach how to make and/or use is proper (In re Marzocchi, 169 USPQ 367 (CCPA 1971). Factors to be considered in

determining whether a disclosure meets the enablement requirement of 35 U.S.C. 112, first paragraph, have been described in In re Colianni, 195 USPQ 150, 153 (CCPA 1977), have been clarified by the Board of Patent Appeals and Interferences in Ex parte Forman, 230 USPQ 546 (BPAI 1986), and are summarized in In re Wands (858 F2d 731, 737, 8 USPQ2d 1400, 1404 (Fed Cir. 1988). Among the factors are the nature of the invention, the state of the prior art, the predictability or lack thereof in the art, the amount of direction or guidance present, the presence or absence of working examples, the breadth of the claims, and the quantity of experimentation needed. The instant disclosure fails to meet the enablement requirement for the following reasons:

9. *The nature of the invention:* The claims are drawn to methods of generating a differentiated neural cell population from primate pluripotent stem cells wherein the differentiated neural cell population comprises at least about 60% dopaminergic neurons or at least about 30% serotonergic neurons.

10. *The state of the prior art and the predictability or lack thereof in the art:* The prior art provides considerable guidance for derivation of nestin positive and NCAM positive neural stem cells from pluripotent stem cells, and for the differentiation of such cells into dopaminergic neurons and serotonergic neurons. As noted in the rejections of claims under 35 U.S.C. 103(a) in the office action mailed 09/21/2007 and herein, the instant claims recite method steps that are known in the art. Culturing pluripotent mouse embryonic stem cells to form embryoid bodies, followed by culture in serum-free ITSFn medium to select nestin+ neural stem cells (instant claims 12-22), as well as expansion and differentiation of the selected neural progenitor cells in the presence of insulin, selenite, transferrin, putrescine, GDNF,

dbcAMP (instant claims 26-28 and 31) is taught by Rolletschek *et al*., Mechanisms of Development (2001) 105:93-104, in section 4.1, page 101 left column, 2nd paragraph (of record). Rolletschek reported a maximum of 85% nestin-positive cells, which declined with longer culture (p. 94, paragraph bridging the columns). These cells could be differentiated to yield populations of about 40% dopaminergic neurons (Fig. 4). WO 200188104 (Carpenter 2001; of record) teaches a method wherein embryoid bodies were formed in suspension and then allowed to adhere to a fibronectin coated substrate, NCAM+ were sorted, and then cultivated in medium containing N2 and B27 supplements, each of which contain selenite, insulin, and transferrin (p.19, lines 23-35; p.20, lines 6-20; pp.21-22). US 20020068045, (Reubinoff; of record) discloses cultivation of embryoid bodies and selection of neural progenitors in a medium that contained B27 supplement, but with no added fibronectin [0369-0371]. Therefore, each of these relevant prior art documents teaches the use of serum-free media supplemented with selenite, insulin, and transferrin (e.g. N2 or B27 supplement mixes). The use of these supplements is routine in the art in the culture of neural cells (Carpenter 2001, p. 11, lines 20-24). The relevant art is devoid of guidance as to the effect the absence of selenite, insulin, and transferrin on yield and purity of differentiated neural populations derived from embryonic stem cells.

11. US 20020019046 (Carpenter 2002) teaches that that "direct differentiation", without the formation of embryoid bodies, "can provide a remarkably consistent population of differentiated cells, with less heterogeneity than what is present in a population of embryoid-body derived cells. Example 5 of this disclosure illustrates that the direct differentiation method can be used to obtain populations that are highly enriched for dopaminergic neurons"

[0043]. The disclosed protocol has human embryonic stem cells cultured on laminin (not fibronectin) in the presence of B27 supplement [0204-0206]. Only about 15% of the neurons in the "highly enriched" population expressed tyrosine hydroxylase (Table 5). It is evident from Carpenter 2002 that differentiation without the formation of embryoid bodies alters the yield and purity of differentiated neural populations derived from human embryonic stem cells, as contrasted to methods that permit embryoid body formation.

12. The guidance provided by the prior art is so thorough that claims that merely recite expansion of stem cells, selection of cells that are positive for nestin and NCAM, followed by differentiation in TGF-β3 or interleukin-1β have been rejected as being obvious. The prior art, however, does not provide a basis for predicting that the differentiated neuronal population thus derived would be at least about 60% dopaminergic neurons or at least about 30% serotonergic neurons as recited in the instant claims. Furthermore, in view of the above, the prior art points to unpredictability as to effect of varying critical parameters of the protocol.

13. *The amount of direction or guidance present and the presence or absence of working examples:* Enablement must be provided by the specification unless it is well known in the art. *In re Buchner* 18 USPQ 2d 1331 (Fed. Cir. 1991). The instant specification does indeed teach a method that will yield a differentiated neuronal population comprising at least about 60% dopaminergic neurons or at least about 30% serotonergic neurons (Fig.10, Fig 11). The described method comprises formation of embryoid bodies and cultivation of the embryoid bodies in ITSFn (nestin selection) serum-free defined medium, which is taught to allow for the selection of nestin-positive cells [0114-0118]. The yield of 60% dopaminergic neurons

required immnosorting for NCAM (Fig. 10 vs. Fig 11). Differentiation without embryoid body formation, in the presence of serum, or in the absence of any component of ITFSn medium was not tested.

14. *The quantity of experimentation needed:* It is not clear that any amount of experimentation would result in a method that starts with human embryonic stem cells and yields a differentiated neural cell population comprises at least about 60% dopaminergic neurons or at least about 30% serotonergic neurons, wherein the method does not comprise steps of embryoid formation followed by culture of the embryoid bodies in serum-free ITSFn medium to select neural progenitor cells.

Conclusion

15. No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Daniel C. Gamett, PhD., whose telephone number is (571)272-1853. The examiner can normally be reached on 8:30-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Manjunath N. Rao can be reached on 571 272 0939. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Daniel C Gamett/
Examiner, Art Unit 1647